Relative Validity of a Food Frequency Questionnaire with a Meat-Cooking and Heterocyclic Amine Module

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Abstract

The nutrient and heterocyclic amine (HCA) intake of 165 healthy participants was assessed using a self-administered food frequency questionnaire (FFQ), which included a meat-cooking practices module. A database containing the HCA [2-amino-3,8-dimethylimadazo [4,5-f] quinoxaline (MeIQx) and 2-amino-1-methyl-6-phenylimadazo [4,5-b] pyridine (PhIP)] composition of various types of meat, cooked by different methods and to varying degrees, was developed and validated in conjunction with this module. The relative validity of dietary and HCA intake estimated by the FFQ was investigated using multiple food diaries (3 sets of 4 nonconsecutive day diaries completed over a 3-month period) as the reference method. Crude correlation coefficients of HCA intake assessed by the FFQ and food diaries were 0.43 [95% confidence interval (CI) 0.30–0.55] for MeIQx and 0.22 (95% CI 0.07–0.36) for PhIP intake. Deattenuated correlations were 0.60 (95% CI 0.49–0.69) and 0.36 (95% CI 0.22–0.49), respectively. Absolute MeIQx and PhIP intake was, however, underestimated by the FFQ (21.9 and 78.1 ng/day) compared with the food diaries (34.9 and 263.8 ng/day). The FFQ underestimated total red meat intake, the percentage of consumers, and the median intake of roast/baked and microwaved red meat. PhIP intake was severely underestimated by the FFQ and was most likely because of an underestimation of the percentage of people who cooked chicken using PhIP-producing cooking methods such as broiling and pan-frying. Additionally, the FFQ overestimated the percentage of consumers of baked chicken, a cooking method that produces less PhIP. In conclusion, although the FFQ and meat module underestimated absolute MeIQx and PhIP intake, its ability to rank individuals according to intake was acceptable.

Introduction

Numerous studies have demonstrated an association between red meat intake and the etiology of various cancers (1–8). A group of mutagenic and carcinogenic compounds called heterocyclic amines (HCAs), formed through pyrolysis of amino acids and creatine in meat using high temperature cooking techniques (9), have been implicated in this association. HCAs are among the most potent mutagens tested by the Ames *Salmonella* test (10) and have demonstrated their carcinogenicity in animals producing tumors in a variety of organs (11, 12). Assessment of dietary HCA intake is challenging as the HCA composition of meat varies according to cooking technique, temperature, cooking time, and meat type (13–16). Epidemiological studies have tried to overcome these difficulties using surrogate markers of HCA intake such as the method of cooking, surface browning, total cooking time, and gravy intake (1, 17, 18). These studies produced suggestive but inconsistent links between HCA intake and cancer risk. Therefore, to investigate the role of HCAs in cancer etiology, improvements in the assessment of HCA intake are required. This study describes the development and validation of a meat-cooking practices module, which was included in a food frequency questionnaire (FFQ), to assess dietary HCA intake with greater accuracy. A database of the HCA content of commonly consumed meats, subjected to representative cooking practices, was developed and used in conjunction with this meat-cooking practices module. The aim of the present study therefore was to assess the relative validity of the newly developed meat-cooking practices module and to assess the absolute dietary HCA intake in a healthy population. Dietary intake of the two most abundant HCAs was considered: 2-amino-3,8-dimethyl-imadazo[4,5-f]quinoxaline (MeIQx) and 2-amino-1-methyl-6-phenyl-imidazo[4,5-b]pyridine (PhIP).

Materials and Methods
Study Population

Subjects were controls participating in a breast and prostate cancer case-control study (Genetics, Environment and Metabolism conducted at Bethesda National Naval Medical Center), Bethesda, Maryland, from August 1993 to April 1995. Male controls were recruited through referrals from clinic physicians and self-referrals generated by recruitment flyer. Those individuals who were present at prostate cancer screening days and who had a negative prostate biopsy and were therefore found to have no indications of prostate cancer were included in the male control group. Female controls were recruited from spouses of male controls seen in the urology clinic and from additional clinic waiting room areas at Bethesda National Naval Medical Center. Female controls were ages ≥40 years and were not taking systemic estrogens. Males and females with a previous diagnosis of malignancies (with the exception of skin cancer *in situ*), a history of heart disease, diabetes mellitus, or liver

Table 1 Demographic details of 165 study participants

	Number (%)
Gender	
Male	118 (72%)
Female	47 (28%)
Race	
White	153 (93%)
Black	5 (3%)
Other	7 (4%)
Age (yrs)	
<60	35 (21%)
60–69	60 (36%)
>69	68 (41%)
Unknown	2 (1.2%)

disease were excluded. Demographic details of the study participants are shown in Table 1.

Data Collection

FFQ. Study participants completed a self-administered, modified version of the 1992 version of the 100-item Health Habits and History Questionnaire (FFQ), which assessed usual dietary intake over the previous year (19). An interviewer-administered meat-cooking practices module was completed to assess the consumption of 23 meat/poultry and fish items using a matrix format similar to the 100-item FFQ. The questionnaire collected information on cooking methods; embedded questions assessed how well the meat was cooked. The study participants estimated whether their portion size was small, medium, or large relative to the standard portion size indicated for each food listed in the FFQ and meat module.

Information on how well meat was cooked was obtained for five red meat items, including hamburger/cheeseburger, beefsteak, pork chops/ham steaks, sausage/hotdogs, and bacon using photographs. Four photographs indicating both the internal coloring and external browning were used to estimate how well hamburgers and steak were cooked (16). Three photographs were used to determine how well pork chops/ham steaks, sausage/hotdogs, and bacon were cooked (15). Information regarding the cooking methods typically used was also collected for each of these five red meat items and for beef and pork roast. This information indicated whether the meat was pan-fried, grilled/barbecued (by placing it on a grid over coals, open fire, or ceramic briquettes heated by gas), oven-broiled (by placing it below the heat source), baked/roasted, or microwaved. For those red meats that are typically cooked in a standard way, for example beef stew, meat loaf, liverwurst, and luncheon meat, no cooking method information was collected. Information on chicken, turkey, and fish intakes, categorized by the method of cooking used and the degree to which it was cooked, was collected in the same way.

Meat intake was calculated using the frequency of consumption and portion size indicated by the respondent in the FFQ. Red meat intake (g/day) was categorized as either medium/rare, well done, or very well done. Variables were also created for each of the cooking techniques mentioned. Therefore, the daily gram intake of each of the red meat categories was calculated based on the cooking technique used and on how well it was cooked. The FFQ data were processed using the dietary analysis software for the Health Habits and History Questionnaire (20) and was modified by the addition of the HCA database (CHARRED).

Multiple Food Diaries. The participants were instructed in-person at the hospital to complete three sets of 4 nonconsecutive day food diaries. Emphasis was placed on describing the type of meat eaten, including meat in mixed dishes, the preparation method, cooking time, and temperature used. The photographs described earlier were also used with the multiple food diaries to estimate the degree to which meat was cooked both internally and externally (15, 16). Measuring guides such as measuring cups, spoons, glasses, bowls, and a ruler were used to improve estimation of portion sizes consumed. Participants recorded their intake on four nonconsecutive days within a 1-week time frame on three separate occasions over the course of 3 months. A post interview session was conducted by telephone to obtain additional details and for clarification. The Food Intake Analysis System (version 2.1) designed by the University of Texas was used to code and analyze the food diary data for the macronutrient, micronutrient, and HCA (MeIQx and PhIP) intake of each participant. This analysis included the calculation of meat intake categorized by cooking method and degree of cooking.

Statistical Analysis. Statistical analysis was carried out using SPSS (version 9). For each nutrient or measure of HCA or meat intake, an estimate of the relative validity of the FFQ was obtained by calculating the correlation coefficient between the estimate of intake assessed by the FFQ and the average of the 12 days of food diaries. Energy-adjusted correlations were calculated using the nutrient density model, and nutrient and HCA correlations were deattenuated to adjust for measurement error (21). The mean difference in intake assessed by both methods was tested for significance using a *t* distribution of paired observations. To examine the existence of systematic bias, the mean difference in intake assessed by both dietary assessment methods was calculated for each nutrient and for MeIQx and PhIP intake. To exclude the possibility of a nonconstant bias, *i.e.,* a bias which depends on the level of intake, the difference between the two measurements (x = recalls) and (y = FFQ) for each individual ($d_i = x_i - y_i$) and the mean values [$m_i = (x_i + y_i/2)$] were computed as described by Grootenhuis *et al.* (22). The relationship between these parameters was studied by regression analysis, as advocated by Altman and Bland (23). The ability of the FFQ and meat cooking module to classify individuals into the same quintile of nutrient, MeIQx and PhIP intake, compared with the multiple food diaries was assessed to evaluate the agreement between both dietary assessment methods.

Results

A comparison of the nutrient intake assessed by the FFQ and the multiple food diaries is shown in Table 2. Results are shown for those nutrients that are commonly associated with meat intake, and crude Pearson correlation coefficients ranged from $r = 0.30$ [95% confidence interval (CI) 0.16–0.43] for zinc to $r = 0.52$ (95% CI 0.41–0.62) for fat intake. Energy adjustment decreased the correlation for protein, iron, and zinc intake and increased the correlation for fat and carbohydrate intake. Deattenuation increased correlations for all nutrients increasing the range from 0.35 (95% CI 0.21–0.48) for protein intake to 0.69 (95% CI 0.60–0.76) for carbohydrate intake. The FFQ underestimated energy, protein, fat, carbohydrate, zinc, and iron intake (Table 2). Regression analysis demonstrated that the differences between the individual pairs of intake estimates were significantly related to the means for the majority of nutrients. Low intakes were overestimated, and high intakes were underes-

Table 2 Selected nutrient intake assessed by the food frequency questionnaire (FFQ) compared with intake assessed by multiple food diaries for the total population (n = 165) using crude, energy adjusted, and deattenuated correlation coefficients (95% confidence interval)

	FFQ Mean (SD)	Food diaries Mean (SD)	Crude correlation	Energy adjusted[a]	Deattenuated[b]
Energy (Kcal)	1743 (701)[c]	1992 (520)	0.45 (0.31–0.56)	0.45 (0.31–0.56)	0.47 (0.35–0.58)
Fat (g)	61.5 (33.4)[c]	70.8 (25.7)	0.52 (0.41–0.62)	0.62 (0.51–0.70)	0.66 (0.56–0.74)
Carbohydrate (g)	216.0 (89)[c]	253.9 (71.4)	0.42 (0.29–0.54)	0.63 (0.53–0.71)	0.69 (0.60–0.76)
Protein (g)	66.3 (27.1)[c]	79.7 (20.0)	0.47 (0.34–0.58)	0.32 (0.17–0.45)	0.35 (0.21–0.48)
Iron (mg)	13.3 (5.3)[c]	17.2 (6.9)	0.40 (0.26–0.52)	0.38 (0.24–0.50)	0.41 (0.28–0.53)
Zinc (mg)	11.2 (10.5)	12.3 (5.2)	0.30 (0.16–0.43)	0.18 (0.03–0.32)	0.42 (0.30–0.57)
MeIQx (ng/day)	21.9 (26.4)[c]	34.9 (36.1)	0.43 (0.30–0.55)	0.41 (0.28–0.53)	0.60 (0.49–0.69)
PhIP (ng/day)	78.1 (142)[c]	263.8 (513)	0.22 (0.07–0.36)	0.22 (0.07–0.36)	0.36 (0.22–0.49)

[a] Nutrient density model.
[b] Correcting for the attenuating effect of within-person error in dietary intake: true correlation = observed correlation X $\sqrt{1 + [(\text{within variance/between variance})/12]}$; Ref. 21].
[c] The FFQ value is >10% different from the value from the food diaries.

timated for energy, protein, carbohydrate, and fat intake resulting in a regression equation $y_i - x_i = \alpha + \beta (x_i + y_i/2)$ in which β was equal to -0.25, -0.21, -0.33, and -0.26 respectively. Conversely, low iron intake was underestimated, whereas high intake was overestimated ($\beta = 0.22$).

Table 2 shows also the HCA intake assessed by both dietary assessment methods. Crude Spearman rank correlation coefficients for MeIQx and PhIP intake assessed by both dietary assessment methods were $r = 0.43$ (95% CI 0.30–0.55) and $r = 0.22$ (95% CI 0.07–0.36), respectively. MeIQx and PhIP intake assessed by the FFQ (21.9 and 78.1 ng/day) were, however, significantly underestimated ($P < 0.001$) compared with estimates assessed by the food diaries (34.9 and 263.8 ng/day). Deattenuation increased correlations to 0.60 (95% CI 0.49–0.69) and 0.36 (95% CI 0.22–0.49) for MeIQx and PhIP intake, respectively. Regression analysis demonstrated that the differences between the individual pairs of intake estimates for MeIQx and PhIP were significantly related to the mean where low MeIQx and PhIP intake was underestimated, and high intake was overestimated ($\beta = 0.29$ and 0.47, respectively).

Classification of individuals by the FFQ into the same or adjacent quintile as by the multiple diaries for nutrients ranged from 61.8% for zinc intake (mg/day) to 78.2% for carbohydrate intake (percentage of energy; Table 3). Classification of individuals by the FFQ into the same or adjacent quintile as by the multiple diaries for HCA intake (ng/day) was 70.3% for MeIQx

and 63% for PhIP intake (Table 3). Misclassification from one quintile to the extreme quintile occurred in <6% of the group for all nutrients and for MeIQx and PhIP intake (Table 3).

Table 4 shows estimates of meat intake (g/day) among consumers assessed by the FFQ and the multiple food diaries. Although absolute red meat intake was underestimated by the FFQ compared with the food diaries (19.8 *versus* 36.6 g/day), the energy-adjusted correlation was 0.40 (95% CI 0.25–0.53). Estimates of bacon, hamburger, and chicken intake assessed by the FFQ were comparable with estimates assessed by the food diaries. However, pork, beefsteak, and sausage intake were underestimated by the FFQ.

Table 5 shows meat intake (g/day) assessed by both methods and according to the method of cooking used. The FFQ underestimated the percentage of consumers of roast/baked (16 *versus* 40%) and microwaved red meat (35 *versus* 50%). Pan-fried and grilled/barbecued red meat intake assessed by the FFQ was reasonably well correlated with estimates from the reference method. The FFQ underestimated, substantially, the percentage of consumers of grilled/barbecued chicken (24 *versus* 42%) and pan-fried chicken (11 *versus* 27%) compared with the reference method. In addition, the FFQ overestimated the percentage of consumers of baked chicken (85 *versus* 64%). Although total chicken intake, assessed by the FFQ, was reasonably well correlated with estimates from the reference method, the FFQ was not good as estimating intake of chicken according to the method of cooking used, apart from perhaps, baked chicken (median intake 14 *versus* 15 g/day, energy adjusted correlation 0.29, 95% CI 0.10–0.45).

Table 6 shows estimates of meat intake (g/day) according to the degree to which it was cooked. Total red meat intake, beefsteak, bacon, and chicken, cooked well/very well done was reasonably well correlated with intake assessed by the reference method. Chicken, which is cooked rare/medium, was also well correlated; however, the number of consumers in this group was very low ($n = 16$).

Discussion

The present study is the first study to assess the relative validity of a FFQ, which included a newly developed meat-cooking practices module, designed primarily to assess HCA and meat intake. Dietary intake of the two most abundant HCAs was considered: MeIQx and PhIP. In addition to HCA intake, the meat-cooking module provides descriptive information about the typical method of cooking used and the degree to which meat is typically cooked.

Table 3 Ability of the food frequency questionnaire (FFQ) to classify individuals into the same or adjacent quintile of nutrient intake as the multiple food diaries (n = 165) expressed as percent of subjects (number of subjects are in parentheses)

Nutrient	Same quintile % (no.)	Same or adjacent quintile % (no.)	Grossly misclassified[a] % (no.)
Energy (Kcal)	27.9 (46)	66.7 (110)	3.0 (5)
Total Fat (%E)	38.2 (63)	76.4 (126)	2.4 (4)
Carbohydrate (%E)	38.8 (64)	78.2 (129)	1.2 (2)
Protein (%E)	32.1 (53)	64.8 (107)	4.2 (7)
Total Fat (g)	32.7 (54)	76.4 (126)	0 (0)
Carbohydrate (g)	29.7 (49)	69.1 (114)	1.8 (3)
Protein (g)	35.2 (58)	72.1 (119)	1.8 (3)
Iron (mg)	22.4 (37)	73.9 (122)	1.8 (3)
Zinc (mg)	32.0 (53)	61.8 (102)	4.2 (7)
MeIQx (ng/day)	25.5 (42)	70.3 (116)	4.2 (7)
PhIP (ng/day)	29.1 (48)	63 (104)	5.5 (9)

[a] Quintile 1 on food diary *versus* quintile 5 on the FFQ or vice versa.

Table 4 Meat consumption (g/day) among consumers assessed by the food frequency questionnaire (FFQ) and multiple food diaries

Meat	FFQ Median (range)	Food diaries Median (range)	Crude correlation (95% confidence interval)	Energy adjusted[a]
Bacon	2.1 (0.3–13)	2.4 (0.1–41)	0.35 (0.21–0.48)	0.53 (0.41–0.63)
Hamburger	7.0 (1.4–40)	9.9 (1.8–48)	0.25 (0.11–0.39)	0.20 (0.05–0.34)
Chicken	27.4 (1.6–99)	23.8 (1.8–96)	0.28 (0.13–0.42)	0.31 (0.16–0.44)
Pork	5.9 (1.5–25)	13.9 (0.6–129)	0.21 (0.06–0.35)	0.28 (0.13–0.42)
Beefsteak	9.2 (1.9–48)	16.6 (3.4–76)	0.21 (0.06–0.35)	0.37 (0.23–0.50)
Sausage	4.5 (1.8–24)	6.9 (0.5–69.7)	0.12 (−0.03–0.26)	0.02 (−0.13–0.17)
Total red meat	19.8 (1.4–125)	36.6 (1.3–142)	0.46 (0.32–0.58)	0.40 (0.25–0.53)

[a] Nutrient density model.

The results of this study indicate that the FFQ and meat-cooking module provide a reasonably good estimate of dietary MeIQx exposure, which is an essential methodological step to assess the relationship between intake and disease occurrence. However, PhIP intake, which was severely underestimated by the FFQ, was poorly correlated with intake assessed by the reference method. Energy, protein, fat, and carbohydrate intakes were compared between the two dietary methods in order to assess the relative validity of the FFQ for more commonly studied intake variables. In addition, some of the micronutrients commonly found in meat such as zinc and iron were compared. Energy adjusted correlation coefficients ranged from 0.18 (95% CI 0.03–0.32) for zinc to 0.63 (95% CI 0.53–0.71) for carbohydrate intake, and these results are comparable with findings from validation studies in Europe and the United States (22, 24–28). The relative validity of the Block FFQ has been extensively studied, and results recently published compared the FFQ to four 24-h recalls in a large sample of men and women. The correlations for macro- and micronutrients were similar to the results shown in the present study (29). Comparisons between studies are, however, crude because of differences in study populations, reference methods, and study design. Energy-adjusted correlations for MeIQx and PhIP intake were 0.41 (95% CI 0.28–0.53) and 0.22 (95% CI 0.07–0.36), respectively. However, deattenuation increased the correlation dramatically for MeIQx (0.60, 95% CI 0.40–0.69) and PhIP intake (0.36, 95% CI 0.22–0.49), indicating that there was substantial measurement error due to random within-person variation. The FFQ underestimated absolute MeIQx (21.9 *versus* 34.9 ng/day) and PhIP intake (78.1 *versus* 263.8 ng/day) compared with the reference method.

Possible reasons for this underestimation include an underestimation of protein intake (66.3 *versus* 79.7 g/day; Table 2) and total red meat intake (19.8 *versus* 36.6 g/day; Table 4). An additional possibility for the underestimation of absolute PhIP intake could be because the FFQ appears to underestimate substantially the consumers of grilled/BBQ chicken (24 *versus* 42%) and pan-fried chicken (11 *versus* 27%), which are PhIP-generating cooking methods. In addition, the FFQ overestimated the consumers of baked chicken (85 *versus* 64%), a cooking method, which generates much less PhIP (Table 5).

Despite this underestimation, the ability of the FFQ to distinguish individuals with high and low MeIQx and PhIP intakes, examined by cross-classification on intake categories (Table 3), was 25.5 and 29%, respectively. These results were comparable with those achieved for macro- and micronutrients studied in previous studies (30).

Dietary assessment of HCA intake is difficult because several parameters, including the amount and type of meat ingested, the frequency of consumption, and the duration, temperature, and cooking method used, must be assessed. For example, the MeIQx concentration of meat is highly variable ranging from nondetectable or very low levels (1.3 ng/g) to 8.2 ng/g (in beefsteak) depending on the method of cooking used and the length of time the meat is cooked (16). The PhIP content is even more variable ranging between 1.9 and 30 ng/g in cooked beefsteak to over 100 ng/g in chicken (16). In general, the HCA content of meat increases as the degree of cooking increases, but the production of individual HCAs (MeIQx or PhIP) is not uniform between cooking methods and degree of cooking. For example, chicken contains much higher levels of PhIP but lower levels of MeIQx compared with

Table 5 Meat intake (g/day) according to cooking method among consumers assessed by the food frequency questionnaire (FFQ) and multiple food diaries

	FFQ		Food diaries			
	Consumers % (no.)	Median (10th, 90th percentile)	Consumers % (no.)	Median (10th, 90th percentile)	Crude correlation[a]	Energy adjusted[b]
Total red meat	88 (145)	20 (6,50)	93 (154)	37 (9,83)	0.46 (0.32–0.58)	0.40 (0.25–0.53)
Pan-fried	70 (115)	6 (2,18)	64 (106)	11 (2,38)	0.41 (0.24–0.56)	0.36 (0.18–0.52)
Grilled/BBQ	60 (99)	11 (3,28)	65 (107)	14 (3,43)	0.35 (0.17–0.52)	0.35 (0.17–0.52)
Ovenbroil	39 (65)	7 (2,23)	38 (62)	12 (2,38)	0.15 (−0.10–0.38)	0.17 (−0.19–0.40)
Roast/Baked	16 (27)	4 (3,14)	40 (66)	11 (4,27)	0.40 (0.02–0.68)	0.39 (0.01–0.67)
Microwaved	35 (58)	3 (1,9)	50 (83)	5 (1,23)	0.26 (0.01–0.49)	0.26 (0.01–0.49)
Total chicken	95 (155)	22 (5,55)	90 (148)	24 (7,56)	0.28 (0.13–0.42)	0.31 (0.16–0.62)
Grilled/BBQ	24 (40)	10 (3,26)	42 (70)	8 (2,32)	0.09 (−0.23–0.39)	0.04 (−0.27–0.34)
Broiled	9 (14)	13 (3,56)	13 (22)	12 (5,26)	−0.42 (−0.79–0.14)	−0.29 (−0.71–0.29)
Pan-fried	11 (18)	8 (3,19)	27 (44)	9 (2,29)	0.44 (−0.04–0.75)	0.33 (−0.16–0.69)
Microwaved	10 (17)	10 (3,42)	33 (55)	8 (3,27)	0.36 (−0.14–0.72)	0.22 (−0.29–0.62)
Roast/Baked	85 (140)	14 (3,45)	64 (106)	15 (4,33)	0.22 (0.03–0.39)	0.29 (0.10–0.45)

[a] Correlation of intake among consumers by both methods only.
[b] Nutrient density model.

Table 6 Meat intake (g/day) assessed by the food frequency questionnaire (FFQ) and by multiple diaries, according to the degree of cooking, among consumers

	% consumers FFQ (no.)	FFQ[a] Median (10th 90th percentile)	% Consumers Diaries (no.)	Food diaries[a] Median (10th 90th percentile)	Crude correlation of intake[b]
Red meat (R/M)[c]	69 (114)	14 (5,34)	81 (133)	20 (2,20)	0.23 (0.05–0.40)
Red meat (W/V)	78 (129)	9 (2,27)	95 (157)	38 (5,74)	0.31 (0.14–0.46)
Beefsteak (R/M)	54 (89)	9 (2,48)	52 (86)	15 (3,61)	0.22 (0.01–0.40)
Beefsteak (W/V)	8 (13)	8 (8,24)	26 (43)	19 (17,20)	0.66 (0.17–0.88)
Hamburger (R/M)	50 (82)	7 (3,40)	22 (37)	9 (5,29)	0.03 (−0.39–0.35)
Hamburger (W/V)	19 (32)	7 (3,24)	37 (61)	8 (3,28)	0.29 (−0.04–0.56)
Bacon (R/M)	5 (8)	2 (0.5,2)	6 (10)	2 (0.7,6)	
Bacon (W/V)	52 (86)	2 (0.5,5)	50 (83)	2 (0.5,6)	0.40 (0.20–0.56)
Chicken (R/M)	10 (16)	28 (5,62)	19 (32)	9 (2,27)	0.66 (0.25–0.87)
Chicken (W/V)	85 (140)	22 (6,55)	88 (145)	23 (7,55)	0.29 (0.13–0.44)

[a] Median (10th, 90th percentile).
[b] Correlation of intake among consumers by both methods only.
[c] R/M, rare/medium, W/V, well done/very well done.

beefsteak (14). Furthermore, the addition of marinades (31) and the use of a microwave cooker to thaw meat will reduce the HCA concentration (32), whereas the cooking technique used may cause variability in HCA concentration, *i.e.,* the number of times the meat is flipped during pan-frying (33).

The parameters assessed in this study included the amount of meat ingested, the cooking method used, and the degree to which the meat was cooked (Tables 4–6). Bacon, hamburger, and chicken intake assessed by the FFQ was similar to the reference method. However, beefsteak, pork, and sausage intake (g/day) were underestimated, which resulted in an underestimation of total red meat intake (Table 4). Using the food diaries as the reference method, the FFQ overestimated the percentage of consumers of pan-fried red meat and underestimated the percentage of consumers of roasted/baked or microwaved red meat (Table 5). Total red meat intake, assessed by the FFQ and according to whether it was cooked rare/medium or well/very-well, was comparable with intake assessed by the reference method. However, agreement between the FFQ and food diaries was more difficult to assess for individual red meat items categorized by the degree of cooking because of a smaller number of consumers within these categories. As a result, the correlations were not deattenuated, and the results are presented here primarily to provide descriptive information. Although total chicken intake and baked chicken were reasonably assessed by the FFQ, agreement between the FFQ and reference method was, however, more difficult to assess for chicken intake cooked by other methods because of the small numbers of consumers in each category.

There are several additions that could improve the ability of the meat module to assess red meat intake with improved accuracy. For example intake of hamburgers, sausages, and bacon should be assessed for eating occasions at home, in restaurants, and in fast food restaurants. Pizza intake was assessed without specifically asking about meat toppings typically chosen. Beef roast, pork roast, and pot roast eaten in sandwiches should be assessed separately to that eaten as part of a meal. As the FFQ underestimated the percentage of consumers of grilled/barbecued chicken and broiled chicken, chicken burgers and chicken eaten at fast-food restaurants should be assessed separately to chicken eaten at home. To assess PhIP intake with more accuracy, the meat-cooking module requires the addition of photographs to assess the degree to which chicken is cooked in the same way as red meat.

Multiple food diaries were chosen as the reference method in this study as they rely minimally on memory and include open-ended responses. In addition, assessment of within and between-person variability in dietary intake was feasible using this method. The food diary, which does not rely on memory recall, is unlikely to share the same random error as the FFQ for assessment of nutrient intake. However, because the same method was used to assess the degree to which meat is cooked (photographs), random error is likely to be similar between the methods, and therefore, correlation coefficients for HCA intake may be overestimated due to a correlation of errors (30). The 12 days chosen for dietary assessment included both weekdays and weekends and were spread over a 3-month period to capture the day-to-day variability of the diet.

Results from this study were used to calculate the degree to which risk estimates, associated with an increased intake of HCAs, are attenuated by measurement error associated with the assessment of dietary HCA intake (Table 7). Expected attenuated relative risks, as a result of misclassification, of MeIQx, PhIP, total red meat, and total chicken intake assessed by the FFQ, indicate that true relative risks associated with an increased intake of HCAs are much greater than was previously thought (Table 7).

The HCA database is generalizable to those countries where meat is cooked using the same techniques and the same preparation methods as the United States. The database may not, however, provide reasonable estimates in those countries using alternative methods of cooking. It is also thought that a biochemical measure of HCA intake may be the only feasible way to estimate intake or exposure due to the large variability in HCA composition present as a result of variability in cooking methods used. A biomarker, which could serve as an independent measure of HCA intake, which is unbiased, independent of dietary assessment error and could account for variability between foods, would be ideal. Biomonitoring of HCA metabolites in human urine has revealed interesting results where dietary MeIQx and its metabolite N-OH-MeIQx-N²-glucuronide excreted in urine were significantly correlated ($r = 0.49$;

Table 7 Expected attenuated relative risks as a result of misclassification of MeIQx, PhIP, total red meat and total chicken intake assessed by the FFQ

	Hypothetical relative risk		
	1.5	2.0	2.5
MeIQx	1.01	1.02	1.03
PhIP	1.02	1.03	1.03
Total red meat	1.02	1.04	1.05
Total chicken	1.00	1.01	1.02

$P < 0.0001$). However, there is a large intraindividual variability in the amount of ingested dose recovered in a 12-h urine collection (34). In addition, the half-life of the MeIQx metabolite, N-OH-MeIQx-N^2-glucuronide, is ~12 h and reflects dietary intake from the very recent past. This marker will therefore not provide an accurate assessment of usual HCA intake or exposure. Additional research in this area may demonstrate that the average concentration of multiple urinary samples captures the day-to-day variability in MeIQx intake adequately to reflect usual exposure or long-term intake.

In conclusion, although the FFQ and meat module underestimated absolute MeIQx and PhIP intake, its ability to rank an individual according to intake was acceptable and can therefore be used as an effective method in epidemiological studies. The module, which requires ~15 min to complete, is therefore a viable option for inclusion in other studies. Assessment of the relative validity of the meat module to assess intake of each individual meat type, each method of cooking, and by the degree to which the meat was cooked, was not possible because of the small number of consumers in each stratum. However, several improvements, described above, could improve the meat module's ability to assess an individual's absolute MeIQx and PhIP intake for future studies.

Acknowledgments

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